The annual incidence of Listeria monocytogenes infection among pregnant women with abortion and premature birth effects in Kirkuk city, Iraq

Incidencia anual de infección por Listeria monocytogenes entre mujeres embarazadas con efectos de aborto y parto prematuro en la ciudad de Kirkuk, Irak

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Abstract

Background: Listeriosis is a severe infection that causes miscarriage and stillbirth in pregnant women. It is common in developing nations with unsanitary living circumstances.

Aims of the study: The study's goals are to isolate and identify Listeria monocytogenes from the placenta and cervix of women who have had many abortions and investigate the link between this germ and occurrences of miscarriage and premature birth.

Examine their ability to create virulence factors, and then investigate the susceptibility and resistance of bacteria to various antibiotics.

Method: 86 cervical and placental swabs were obtained from women who had an abortion or were in the early stages of labor. Listeria monocytogenes were isolated and identified, and the bacterium's virulence factors were investigated and a susceptibility test to common antibiotics.

Results: The results showed that 34 of the 86 swabs tested positive for L. monocytogenes, accounting for 39.53 percent of the total. It was divided amongst 29 isolates from cervical (pap smear) samples (43.93 %) and five isolates from 20 placental swabs (25%). The age group (26-35 years) had the highest rate of miscarriage or early deliveries (41.86%). The current findings revealed that the most significant number of samples were taken from women who had stillbirth 33 (38.37%) compared to women who had abortions and premature births 22 (27.915%), 29 (33.72%), and so on. There was no link between chronic disorders like hypertension or other conditions like gestational diabetes and listeriosis susceptibility, as most of the cases were free of these conditions (26.74%). The majority of the positive cases were from uneducated people who lived in a rural area with a common standard of living (27.91%). The bacterium's virulence factors, such as the synthesis of Protease, Lipase, and Lecithinase enzymes and its slime layer, were investigated. The susceptibility of the listeria to certain medications was also investigated, with all isolates showing resistance to the antibiotic Nalidixic acid (100%) but sensitivity to the antibiotics Ampicillin and Chloramphenicol (100%) and varying sensitivity to the rest.

Keywords: Listeria monocytogenes, virulence factor, abortion, miscarriage, stillbirth, risk factors, Antibiotics.

Resumen

Antecedentes: la listeriosis es una infección grave que provoca aborto espontáneo y muerte fetal en mujeres embarazadas. Es común en países en desarrollo con condiciones de vida insalubres.

Objetivos del estudio: Los objetivos del estudio son aislar e identificar Listeria monocytogenes de la placenta y el cuello uterino de mujeres que han tenido muchos abortos e investigar el vínculo entre este germen y los casos de aborto espontáneo y parto prematuro.

Examine su capacidad para crear factores de virulencia y luego investigue la susceptibilidad y resistencia de las bacterias a varios antibióticos.

Método: Se obtuvieron 86 hisopados cervicales y placentarios de mujeres que abortaron o se encontraban en las primeras etapas del trabajo de parto. Se aisló e identificó Listeria monocytogenes, y se investigaron los factores de virulencia de la bacteria y se realizó una prueba de susceptibilidad a los antibióticos comunes.

Resultados: Los resultados mostraron que 34 de los 86 hisopos dieron positivo para L. monocytogenes, lo que representa el 39,53 por ciento del total. Se dividió entre 29 aislamientos de muestras de cuello uterino (panicolau) (43,93%) y 5 aislamientos de 20 hisopados de placenta (25 %). El grupo de edad (26-35 años) presentó la mayor tasa de abortos espontáneos o partos prematuros (41,86%). Los hallazgos actuales revelaron que el número más significativo de muestras se tomó de mujeres que tuvieron muerte fetal 33 (38,37%) en comparación con mujeres que tuvieron abortos y partos prematuros 22 (27,915%), 29 (33,72%), y así sucesivamente. No hubo relación entre trastornos crónicos como la hipertensión u otras condiciones como la diabetes gestacional y la susceptibilidad a la listeriosis, ya que la mayoría de los casos estaban libres de estas condiciones (26,74%). La mayoría de los casos positivos procedían de personas sin educación y vivían en una zona rural con un nivel de vida ordinario (27,91%). Se investigaron los factores de virulencia de la bacteria, como la síntesis de las enzimas proteasa, lipasa y lecitinasa y su capa mucosa. También se investigó la susceptibilidad de la listeria a ciertos medicamentos, mostrando todos los aislamientos resistencia al antibiótico ácido nalidíxico (100%) pero sensibilidad a los antibióticos ampicilina y cloranfenicol (100%) y sensibilidad variable al resto.

Palabras clave: Listeria monocytogenes, factor de virulencia, aborto, aborto espontáneo, muerte fetal, factores de riesgo, antibióticos.

Introduction

Introduction

The Listeria genus incorporates several Gram-positive bacteria with a low GC content, facultative anaerobic bacilli tiny, non-spores, do not possess capsular, and motile at temperatures between 10-25°C[1]. Because of its ability to secrete two types of proteins, L. monocytogenes is a common opportunistic bacterium that is dangerous to humans and animals, causing death. It is also considered an intracellular bacterium that can invade different cells and is responsible for severe infections in both humans and animals. Inositol-Specific Phospholipase C and Listeriolysin O[2-4].

L. monocytogenes spread widely in soil and rural environments, so it is one of the most important contaminants of raw food materials used to manufacture ready-to-eat foods such as milk and its derivatives. The bacteria are characterized by their ability to tolerate high salt concentrations and a high PH level, in addition to their ability to survive and reproduce at temperatures as low as 4°C[5]. L. monocytogenes causes infections known as Listeriosis. The infection usually occurs through eating contaminated food. It moves from the intestine and enters the blood circulation, causing more systemic infections than intestinal infections, including Meningitis, the most common clinical manifestation of Listeriosis[6-8]. Furthermore, there are several species of Listeria; only L. monocytogenes are the pathogen to humans[9, 10]. These bacteria also cause Endocarditis, Hepatitis, Pneumonia, Arthritis, and other systemic infections[11-13], and the people most exposed to infection with Listeriosis are the elderly, Diabetics, immunosuppressed, and people with liver failure, as well as pregnant women and newborns who are more sensitive to Listeriosis, as a result of the deficiency in cellular immunity during pregnancy and childbirth[14, 15]. Pregnant women are at risk of contracting Listeriosis. That infection can be transmitted to the uterus and lead to severe complications, including amnionitis, preterm labor, and cases of spontaneous abortion or stillbirths. The infection is transmitted to the fetus and leads to the birth of children infected with Listeriosis [16, 17]. Thus, due to the significance of the matter, the specific objective of this study was:

1- Isolation and diagnosis of L. monocytogenes from women who had miscarriages and premature labor, based on their biochemical and culture tests.

2- Determining the infection rates with the germ and studying the relationship between repeated isolation of the germ with miscarriage and premature birth cases.

3- To evaluate the ability of bacteria to produce Lipase, Lecithinase, and Protease enzymes, in addition to the ability of bacteria to produce mucus.

4- To implement and validate the sensitivity and resistance of bacteria to several antibiotics.

5- To investigate the role of some risk factors on the extent of the exacerbation and progression of the pathological condition of the pregnant mother.

Methods

The study included collecting 86 swabs of disease cases from February 2019 to June 2019 that included Pap smears from pregnant women who had miscarriages or premature births whose ages ranged between 24-46 years old with clinical symptoms of vaginitis and cervicitis diagnosed by specialist doctors of Kirkuk General Hospital.

Samples were taken from the vaginal area up to the cervix using a sterile cotton swab and then placed in Stuart's Transport medium. The swabs were delivered to the laboratory as quickly as possible to avoid drying the sample. Placenta swabs were collected after birth from premature births or direct miscarriages using a sterile cotton swab. A swab was taken from several areas of the placenta and then placed in the carrier mentioned above medium. All samples were transferred to the laboratory for development and conducting isolation and diagnostic tests.

The cotton swabs were seeded on Blood agar, two culture plates for each sample, one of them was incubated at 37 m for 24 hours, using a specific incubator that provides 5% of CO2, while the other culture plate was incubated at temperatures four °C for 2-5 days, with the emergence of growth observed daily.

The ability of bacteria to grow at different temperatures, including (4°C) and (37°C for 24 hours) and (45°C for seven days) was studied by inoculating tubes of Blood Broth Base with young colonies and incubating them at temperatures (4°C) for a period of Up to 21 days, during which the growth is examined daily. The ability of bacterial isolates to tolerate high salts was determined by using N. Broth with the addition of NaCl at a concentration of 6.5% and 10%, then inoculating each concentration with bacteria at 37°C for 24-48 hours[18,19]. The beta-hemolytic bacteria that grew on the center of the blood agar, ranging in diameter from 0.5-1.5 mm translucent to gray[20] , were diagnosed as L. monocytogenes and then inoculated on the Oxford Listeria Selective agar, where they incubated at 37°C for 48 hours aerobically[21] , then growth was observed where the colonies of L. monocytogenes were green-brown surrounded by a black band as a result of the degradation of esculin[22]. A test for the ability of L. monocytogenes to move was performed by the Wet Mount Method and the culture method on the media for the motion test , and many biochemical tests were performed to ensure the purity of the bacterial isolates [20, 23].

Detection of some virulence factors

1- Lecithinase and Lipase Production test. Inoculation of the egg yolk dens with a sample of the young colony and incubated at 37°C for a period of 24 - 48 hours up to eight days. The production of lecithinase enzyme production was indicated by the appearance of clear areas around the colonies. While the ability of bacteria to produce lipase enzyme was detected by immersing the medium in an amount of saturated copper sulfate solution for 20 minutes, the appearance of a blue-green color at the sites of lipolysis indicates the activity of Lipase enzyme[24].

2- Casein Hydrolysis test (Protease). The bacteria grown on a particular plate were incubated at 37°C for a week. The plates were then examined for clear areas of enzyme degradation[25].

3- Slime Production Test: The tubes of the liquid medium of trypticase soya were inoculated with the young colonies and then incubated at 37°C for 24 hours, after which the contents of the tubes were poured, and drops of safranin dye were added to them and left for a minute and then placed upside down on the filter paper to get rid of the dye, The appearance of the dye on the walls of the inner tube is evidence of a positive test[26].

Antibiotic sensitivity test

Seven types of antibiotics were used to test for the sensitivity of L. monocytogenes to antibiotics. The modified Bauer-Kirby method[27], adopted by the World Health Organization[28,] was applied where included inoculating the medium of Muller-Hinton agar with the bacterial suspension and spreading on the surface of the culture medium. Then the disks were placed using sterile forceps. The plates were incubated at 37°C for 24 hours, then the area of growth inhibition was measured based on the recommendations of the World Health Organization.

Results

The study included 86 swabs, involve 66 pap smears and 20 placental swabs from pregnant women with premature abortions. The number of positive samples for L. monocytogenes reached 34 (39.53%) isolates from the total; it was distributed among 29 isolates of the vagina (Pap smear) (43.94%) of the total 66 isolates and five isolates of the placenta (25%) of the total 20 bacterial isolates, as shown in Table 1.

Table 1. The number and percentage of positive and negative sample

Sample type	The total number	The number of positive samples	percentage 100%	The number of Negative samples	percentage 100%
Pap smear	66	29	43.94	37	56.06
Placenta swab	20	5	25	15	75
Total	86	34	39.53	52	60.46

The current study was characterized by a higher rate of isolation of listeria from the vagina(Pap smear) (43.94%), which is higher than what was found by the researchers Mylonakis et al. [29] as it reached (9%) out of a total of 11 cases of infection with Listeriosis from pregnant women while the percentage of bacteria isolated from the placenta in our study was close(25%) to their results which amounted to 18%. The results of our study are also converging with Nolla-Salas et al.[30,] and Bigrigg et al.[31] who obtained the isolation rate of listeria (44%) from the total uterus (36%), and the placenta (11%). The high rate of isolation of this bacterium from placenta samples is due to its ability to overcome the host's defenses and cross the three barriers of the host, namely the intestinal, blood, and placenta barriers, through their ability to grow and multiply within the host cells[32]. The researcher Parkash et al.[33] showed that L. monocytogenes to transfer from one cell to another gives an idea of how infection occurs across the placenta for newborns compared with other causes of bacteremia in mothers. Also, the presence of L. monocytogenes in a wide range in nature and its contamination of various types of food, and its opportunistic nature in causing infection in pregnant women with weak immunity leads to an increase in its isolation rate[34].

The research questionnaire revealed the role of risk factors associated with Listeriosis, including the role of the age of the pregnant mother in increasing and exacerbating the infection, as the results showed that the highest rate of miscarriage or premature births appeared in the age group (26-35 years) represent (41.86%). Thus, our study confirms the existence of a relationship between infection with the disease and the age group of the pregnant mother, as shown in Table 2, which may be due to aging. A study done by Jones et al. [35] showed that the age factor is one of the essential factors that affect Listeriosis. They confirmed the existence of a relationship between the age of women and the increase in these infections. Contrary to what was mentioned, Essa et al. [36] pointed out that age is one of the factors responsible for the variation in the natural flora of the vagina and causes the periodic appearance of pathogenic microorganisms, and that the high incidence of infections in women of reproductive age and groups (17-25 years) and (26-35 years) is due to the circumstance that this age range represents the early years of marriage in which sexual activity increases and reproductive hormones reach their highest levels compared to the age group 45 years and over.

Table 2. Shows the relationship of incidence of Listeriosis with the age of the pregnant mother

Age of pregnant woman	No. Of cases	Percentage (100%)
17-25	21	24.42
26-35	36	41.86
36-45	29	33.72
Total	86	100

The number of abortions, premature and stillbirths for pregnant women on the incidence of Listeriosis were studied, Table (3) showed convergence in the number and rates of infection in all cases associated with the disease, and this may be due to failure to adhere to the periodic follow-up of the specialist doctor, which increases the chance of exacerbation of the infection, especially in the case of neglect of the pregnant mother in cases of uterine inflammation, recurrent or chronic urinary tract infections [37].

Table 3. The relationship between the number of abortions, premature and Stillbirth with the onset of infection

The condition of the pregnant woman	No. Of cases	Percentage 100%
The number of abortions	24	27.91
The number of premature births	29	33.72
Stillbirth	33	38.37
Total	86	100

The results also showed a significant role in the case that a pregnant woman suffers from chronic diseases, which leads to an increase in the infection of the pregnant woman with Listeriosis Table 4. Among the critical health problems that a woman may face during pregnancy after the age of 45 is the possibility of one Chronic disease, with the risk of pregnancy reaching 50%; this problem is related not only to age but also to hormonal changes[37]. The increasing level of sugar in the blood and urine of pregnant women makes the vagina and urinary tract a suitable environment for the growth of bacteria and yeasts, as many studies indicate that 10-30% of pregnant women get recurrent UTIs due to the invasion and colonization of pathological bacteria in the genitourinary tract of the pregnant woman, which leads to an increase in the rate of abortion and premature birth[38].

Table 4. The relationship of the presence of chronic diseases in the pregnant woman on the appearance of the infection

The condition of the pregnant mother	No. Of cases	Percentage 100%
Heart and arterial disease and presence of hypertension	9	10.47
Gestational diabetes	21	24.42
Osteoporosis	13	15.11
Chronic or recurrent urinary tract infection	20	23.26
Healthy (Disease free)	23	26.74
Total	86	100

Table 5 showed the role of the economic situation, the educational level and the environment in which the pregnant woman is found to have a disease or abortion, as most of cases were due to residents of remote rural areas of hospitals for pregnant women, or as a result of the low scientific or educational level or weak or moderate financial and economic condition and failure to adhere to the vaccination schedule for pregnant women, especially if the pregnant mother is a woman who works in rural areas in sheep and poultry farming, which may be infected or carrying the bacteria in addition to the lack of concern for personal hygiene and not taking into account the minor

pathological symptoms of Listeriosis in pregnant women, which are between fever, vomiting, back pain and headache, all of which lead to an increase in the incidence rate also the cause of abortion may be due to inappropriate environmental conditions in which the pregnant woman lives, hormonal causes, unknown pathologies, or chronic urinary tract infections[36,39].

Table 5. The role of the economic situation, the level of academic achievement, and the environment on the appearance of the infection

The status of the pregnant mother	No. Of cases	Percentage 100%
Educated, from the city environment, with an average standard of living	22	25.58
Uneducated, from the city environment and with a low standard of living	22	25.58
Educated and from a rural environment with an average standard of living	18	20.93
Uneducated and from a rural environment with an average standard of living	24	27.91
Total	86	100

The diagnostic examination results from developing swabs on the blood agar showed the occurrence of complete hemolysis with its morphological characteristics corresponding to what was mentioned by Koneman et al. [20], where the bacteria appeared in the form of bacilli, arranged in pairs, Gram-positive. Sometimes they appear arranged in different shapes such as Y, T, and V. The bacterial isolates were grown on the Oxford Listeria Selective Agar medium. Their colonies appeared in a greenish-brown color. The cultivar medium turned black as a result of the degradation of esculin. Bacterial isolates were also grown on Tryptose Agar with Potassium Tellurite. This bacterium is characterized by its ability to grow on the potassium tellurite medium, reducing it and producing a black compound. Thus its colonies appear black on the medium containing it[8].

The motion screening test was also performed at 25°C as a diagnostic test for L. monocytogenes, as the bacteria have the ability to move at this temperature, and this movement appears similar to the Christmas tree

in an inverted form [40]. The bacteria also lose the ability to move at 37 C. Also, the movement of bacteria was examined using the suspended drop method, and their fluctuating rotational movement was observed upside down according to what was stated by [20, 41,] due to their possession of a group of peritrichous Flagella[42], And many diagnostic tests were performed, Table 6 to ensure the purity of the bacterial isolates.

Table .6. Biochemical Tests use for Diagnosis of L. monocytogenes

The tests	The result
Growth on Oxford Listeria Selective Agar	+
Growth on Tryptose agar with PT medium	+
Catalase enzyme	+
Oxidase enzyme	-
Urease enzyme	-
Gelatinase enzyme	-
Ascholine lysis	+
Indole	-
Methyl red	+
Vogues Proskauer	+
Citrate utilization	-
H2S production	-
O/F of glucose	Oxidation and fermentation of glucose
Decomposition of Hippurate	+
glucose	+
salcin	+
trehalose	+
xylol	-
mannitol	-
maltose	+
CAMP	+

The results of the antibiotic sensitivity test shown in Table 7 to test the effectiveness of antibiotics on L. monocytogenes isolates, as the results showed that all the isolates were 100% sensitive to the antibiotic Chloramphenicol and Ampicillin, followed by Gentamycin, to which the proportion of sensitive isolates was (88.24%), then Erythromycin with a sensitivity rate (79.41%), followed by Trimethoprim (38.24%). In comparison, the sensitivity rate was (29.415%) to the antibiotic Cloxacillin. In contrast, all isolates were resistant to the antibiotic Nalidixic acid. This coincides with the study of the two researchers Prescott and Baggot[43] that the antibiotic Nalidixic acid is used in selective agar to isolate bacteria that are resistant to this antibiotic, as the antibiotic works to inhibit the growth of gram-negative bacteria by its effect on the process of building DNA (DNA). Kalekar et al. [44]. Boucher's[45] study emphasized the importance of using the two antibiotics, Ampicillin and Gentamycin as a first choice in treating Listeriosis. Gómez et al.[9] also showed that the first choice in treating cases of meningitis caused by this bacterium in the newborns is to use the two antagonists, Ampicillin and Gentamycin together. The emergence of antibiotic-resistant isolates can be attributed to the ability of the bacteria to acquire motile genetic factors such as motile plasmids and conjugative transposons[46-50].

Table 7. Sensitivity of L. monocytogenes isolates to antibiotics

Antibiotics	Sensitive isolates		Resist isolates	
	Number	percentage	Number	percentage
Chloramphenicol	34	100	0	0
Ampicillin	34	100	0	0
Gentamycin	30	88.24	4	11.76
Erythromycin	27	79.41	7	20.59
Trimethoprim	13	38.24	21	61.76
Cloxacillin	10	29.41	24	70.59
Nalidixic acid	0	0	34	100

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Conflict of interests
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Ethical Approve
We declare that the study does not need ethical approval

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