**Molecular Detection of *Listeria monocytogenes* among Sudanese Pregnant Women with Pervious Miscarriage in**

**Khartoum State**

**ABSTRACT**

***Back ground****: Listeria monocytogenes* is an emerging food borne pathogen and causative agent of listeriosis. Clinical manifestation of invasive listeriosis is usually severe and includes sepsis and meningoencephalitis.

**Objective**:The objective of the study was to determine the prevalence of *L. monocytogenes* in pregnant women with spontaneous abortions or having a history of spontaneous abortions using PCR.

**Materials and Methods** :In this cross sectional study, a total of 50 samples (vaginal and high vaginal swabs) were collected from 50 women with spontaneous abortion hospitalized in Omdurman Maternity Hospital and Alsudi Hospital in Khartoum State. Each sample was immersed in plastic swab tube containing 5 ml of Tris Hcl buffer (PH 8.0) and transported to research laboratory in Sudan University of Science and Technology as soon as possible for the direct DNA extraction and PCR.

***Result*** *:L. monocytogenes* DNA was detected from 10% samples. 3/50 (6%) and 2/50 (4%) were detected from vaginal and high vaginal swabs respectively. The most affected age group with Listeria infection was 31-36 years old represented 2/19 (10.5%) of aborted women. The most aborted women 3/25 (12%) with *Listeria* infection were had previous abortions within second trimester.

**1-INTRODUCTION:**

Listeriosis is a severe food borne disease that rarely occurs in humans and primarily affects the elderly, persons with impaired immunity, pregnant women and unborn or newborn babies. Although uncommon, compared to other foodborne infections, listeriosis is associated with high mortality (1). It is caused by *L. monocytogenes*, a Gram positive, non-spore forming, facultative intracellular and adaptable environmental bacterium. Although most of bacteria do not grow or grow weakly at temperatures below 4°C, *L. monocytogenes* survives in low temperatures. Therefore, *L. monocytogenes* is an important food born pathogen in ready-to-eat foods that have been refrigerated (2,3) .

*L. monocytogenes* has been found in 10% or more of healthy people, usually in the gut (4). All the 13 serovars of *L. monocytogenes* are reported to cause human listeriosis, but serovars 1/2a, 1/2b and 4b are implicated with most of the cases (5).

Pregnant women are particularly prone to infection. The placenta provides a protective niche for the growth of *L. monocytogenes*, thereby resulting in spontaneous abortions, stillbirth neonatal infection, severe necrotizing hepatitis, placental necrosis and increased risk of post implantation loss (6,7). Latent listeriosis in pregnant women leads to habitual abortions, intrauterine deaths and fetal malformations (8,9).

Listeriosis can occur at any time during pregnancy but it is most often recognized in the third trimester (from 28 weeks of pregnancy) Pregnancy-related cases of listeriosis are classified into early onset and late onset depending on how long after birth the baby starts to develop symptoms. An early onset case is defined as a newborn with symptoms at birth or within 48 h of birth. This is usually attributed to in-utero infection either through ascending spread from vaginal colonization, or more commonly through transplacental transmission from maternal bacteraemia. Late onset is defined as a newborn that develops symptoms 48 hrs after birth. Infection is thought to occur as the baby passes through the birth canal or as a nosocomial infection from another early onset case (10).

The incidence of listeriosis in general population is 0.7 in 100000 but the prevalence is 12 in 100000 in pregnant women (which is a 17-fold increase) (11), this is because during pregnancy the immune system is modulated, with the placenta serving as a protective environment for the growth of the bacterium .

The fetus suffers more damage than the pregnant women, leading to a clinical syndrome known as granulomatosis infantiseptica (12). *L. monocytogenes* causes meningitis and hydrocephalus in children born of infected mothers (13). These reports highlight the importance of the pathogen as a cause of spontaneous abortions and infant mortality(14).

Unlike developed countries, systematic studies done on the association of pathogenic *L. monocytogenes* with spontaneous abortions are lacking, especially in the Sudan.

## Listeria monocytogenes is a Gram positive rod, facultative intracellular,

## foodborne pathogen responsible for cases and out-breaks of listeriosis.

## Earlier studies reported that L. monocytogenes has been isolated from tissue sections of patients in Germany in 1891, from rabbit liver in Sweden in 1911, and from spinal fluid of meningitis patients in 1917 and 1920 (15).

## Listeria was first described in 1926 by Murray et al. who discovered it while investigating an epidemic infection among laboratory rabbits and guinea pigs (16). At that time, it was given the name Bacterium monocytogenes because infection in the animals was characterized by monocytosis. The following year, Pirie isolated an identical bacterium from the liver of several gerbils in South Africa. and proposed the name Listerella hepatolytica for the genus in honor of Lord Lister a prominent surgeon of the time (17). Despite considerable confusion in the nomenclature of the pathogen until 1940, the official name Listeria monocytogenes was adopted in the Sixth Edition of Bergey’s Manual of Determinative Bacteriology (18), and the word “monocytogenes” means monocyte producing, since it produced a typical monocytosis during an illness in the diseased animal

## The first cases of human listeriosis were reported by Nyfeldt in 1929 (19). The increased number of reported cases during the 1980s in several countries, and the evidence of foodborne transmission, turned listeriosis into a recognized foodborne disease (20).

**2- MATERIAL AND METHOD:**

A cross sectional study ,The samples were collected from selected government hospitals in Khartoum State (Omdurman Maternity Hospital and Alsudi Hospital). The genotypic identification of the clinical samples was carried out in Medical Microbiology Research Laboratory of Sudan University of Science and Technology.

The study was carried out form March 2017 to February 2018.

The samples were collected from women with spontaneous miscarriage or having a history of recurrent miscarriage, with different ages and different trimesters, who attended to the selected hospital during the study period.

Fifty clinical specimens (25 vaginal swabs and 25 HVS) were included in this study.

A structured questionnaire was used to collect the data. The questionnaire contains questions on respondent’s socio-demographic characteristics, obstetrical history and other Bio data

The vaginal swab was taken by trained and qualified doctor or sister, the high vaginal swab was taken by trained and qualified doctor or sister with a speculum, by inserting the speculum 3–4 cm into the vagina and rotating the swab with a circular motion, leaving it in the vagina for approximately five seconds. Then the swab was inserted into plastic tube containing 5 ml of Tris Hcl buffer ( PH 8.0). Pellet from these samples were obtained by centrifugation and then re suspended in 2 ml Tris Hcl buffer and stored in falcon tube at -20 °C until used (21).

**Genotypic analysis of bacterial isolates**

**DNA Extraction**

DNA was extracted by thermal lysis (boiling method) (22)

**Polymerase chain reaction (PCR)**

Polymerase chain reaction was carried out using thermo cycler (TECHNE TC-312, UK) (Appendix-I). Specific primer was used for detection of *L. monocytogenes* by conventional PCR.

**Primers**

The primer 5’-TATGTCGGGCAAGCGTTC-3’ and 5’-GCGCTTGCGTGGTAATTC-3’ was used, with product size 281bp.

**Preparation of primers**

**Stock primer**

Centrifugation of primer vial was done firstly then 230μl of sterile DW was added into each primer vial.

**Working primer**

From each stock primer 10μl was dissolved in 90μl of distilled water and stored at -20˚C.

**Master Mix**

Master Mix kits (iNtRON’s Maxime PCR PreMix, Korea) containing all reagents for PCR except water, template and primers was used. Storage of the master mix was carried out at -20˚C.

**Preparation of reaction mixture**

List 1-Preparation of PCR reaction mixture

|  |  |
| --- | --- |
| Reagents | Volume (µl) |
| WFI | 18 |
| Forward primer | 1 |
| Reverse primer | 1 |
| Template | 5 |

**Amplification conditions of PCR**

The amplification was done by using 0.2 PCR eppendorf tubes that subjected to thermo cycler.

|  |  |  |
| --- | --- | --- |
| Phase | PCR conditions | Number of cycles |
| Initial denaturation | 94º C for 2 mines | 1 cycle |
| Denaturation | 94º C for 30 sec | 35 cycle |
| Annealing | 48º C for 30 sec |
| Extension | 72º C for 40 sec |
| Final extentin | 72º C for 5 mines | 1 cycle |

**Gel electrophoresis and visualization under UV light**

**Preparation of 10X TBE**

One Liter of 10x stock solution prepared by dissolving 48.4g Tries base and 55g of boric acid in 40ml of 0.5 M EDTA

**Preparation of 1X TBE**

Ten ml of 10X TEB was diluted by addition of 90ml of de ionized water.

**Preparation of ethidium bromide**

One gram of ethidium bromide was added to 100ml of DW, 1% (10mg/ml) of the solution transferred to dark bottle and stored at room temperature.

**Preparation of agarose gel**

Agarose gel (2%) was prepared by melting 1gm agarose in 50 ml of 1X TBE Buffer using a microwave oven for 1 minute. The melted agarose was allowed to cool to about 50ºC then 2 µl of ethidium bromide was mixed. Agarose gel was poured into gel tray, comb was placed and any air bubbles were removed. After solidification of the gel, the comb was removed and 50ml of 1X TBE buffer was poured into the gel tank to barely submerge the gel.

**Visualization of the DNA products**

The gel casting tray was put into the electrophoresis, tank flooded with 1x TBE buffer just to cover the gel surface, 6μl of PCR products from each sample was added to wells of electrophoreses, 5μl of 100-bp DNA ladder (iNtRON, Korea), was added to the well in each run. The gel electrophoresis apparatus was connected to power supply (100 V, 500 mA, UK). The electrophoresis was carried out at 75Volts for 30 minutes and the gel tray was removed from the electrophoresis apparatus and the buffer was discarded. Then the gel was visualized for DNA bands by U.V transilluminater and photographed (Uvitec –UK) (23).

**Data analysis**

Data was analyzed by using Statistical Package for Social Science Program (SPSS) version (16.0) for frequency and percentage.

**3- Result**

In this study, 50 clinical samples were collected during 2017. Clinical specimens obtained from patients with spontaneous miscarriage hospitalized in Omdurman Maternity Hospital and Alsudi Hospital, including: vaginal and high vaginal swabs.

**3.1. The percentage of positive samples**

The genotypic detection of 50 samples revealed 5 (10%) samples to be positive for *L. monocytogenes* showed in Fig (1). The five isolates from clinical samples were recovered from three vaginal swab samples (6%) and two from high vaginal swab samples (4%).

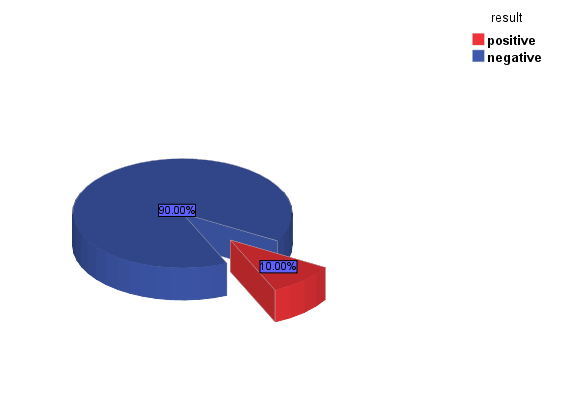


Fig (1) The percentage of positive samples

The majority of positive samples (2 out of 5) were found in the age range 31-36 years (table 1)

**Table (1): Relationship between age and frequency of *L.* *monocytogenes***

|  |  |  |  |
| --- | --- | --- | --- |
| Age | Listeria Spp. |  | Total |
|  | Positive | Negative |  |
| 19-24 yrs | 1  16.7% | 5  83.3% | 6  100.0% |
| 25-30 yrs | 1  5.9% | 16  94.1% | 17  100.0% |
| 31-36 yrs | 2  10.5% | 17  89.5% | 19  100.0% |
| 37-42 yrs | 1  12.5% | 7  87.5% | 8  100.0% |
| Total | 5  10% | 45  90% | 50  100.0% |

Three out of five (60%) of the positive cases were recorded in the second trimester of the gestation period (table.2).

**Table (2): Relationship between gestational age and presence of *L. monocytogenes***

|  |  |  |  |
| --- | --- | --- | --- |
| Trimester | Result |  | Total |
|  | Positive | Negative |  |
| 2nd trimester | 3  12% | 22  88% | 25  100% |
| 3rd trimester | 2  8% | 23  92% | 25  100% |
| Total | 5  10% | 45  90% | 50  100% |

The majority of positive cases of *L. monocytogenes* 4 (80%) were recovered from women having single abortion during their marriage period (table 3).

**Table (3): Relationship between the number of abortion of pregnant women and the infection with *L. monocytogenes***

|  |  |  |  |
| --- | --- | --- | --- |
| No. of abortion | Result |  | Total |
|  | Positive | Negative |  |
| One abortion | 4  14.3% | 24  85.7% | 28  100% |
| Two abortion | 0  0% | 11  100% | 11  100% |
| ≥ 3 abortion | 1  9.1% | 10  90.9% | 11  100% |
| Total | 5  10% | 45  90% | 50  100% |

Most of the positive samples infected with *L. monocytogenes* 3 (60%) were obtained from the vaginal swab (table 4).

**Table (4): Relationship between the location of the sample and the frequency of *L. monocytogenes***

|  |  |  |  |
| --- | --- | --- | --- |
| Type of sample | Result |  | Toyal |
|  | Positive | Negative |  |
| Vaginal swab | 3  12% | 22  88% | 25  100% |
| HVS | 2  8% | 23  92% | 25  100% |
| Total | 5  10% | 45  90% | 50  100% |

The PCR allowed amplification of specific gene of *L. monocytogenes*, the product size is 281bp. Among 50 samples, *L. monocytogenes* was found in 5 samples (Fig 2).

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Fig (2): Agarose gel electrophoresis of PCR amplification products using specific gene to *L. monocytogenes* isolated from clinical human samples.

Lane M: 100bp ladder as molecular DNA marker, Lane 1: Control negative, Lane 2: Negative sample, Lane 3: Positive sample.

**4-Discussion**

Reports of listeriosis from humans in Sudan are uncertain, either because of failure to identify the isolate, its rarity, improper isolation techniques, low incidence rate or lack of awareness.

In the present study, the genotypic detection of 50 samples collected from 50 pregnant women with history of spontaneous abortions revealed 5(10%) samples were identified as *L. monocytogenes*, 3(6%) and 2(4%) for vaginal and high vaginal swab, respectively.

A different frequency of *L. monocytogenes* has been reported from several countries. The prevalence of *L. monocytogenes* in this study (10%) was higher than the earlier reports on the isolation of *L. monocytogenes* from three out of 100 (3%) (24), nine out of 670 (1.3%) (25), two out of 633 (0.3%) (26), four out of 305 (1.3%), one out of 958 (0.1%) (27), five from 300 (1.7%) (28), seven out of 481 (1.5%) (29), 18 out of 2200 (0.81%) (30), and two out of 295 (0.68%) (31).

The variation reported among the studies can be due to differences in the population under study include culture, race, nutrition, ecological region and also laboratorial diagnosis methods and also difference in sites of samples collection.

Also, the result of this study is in agreement with the earlier from ten out of 100 (10%) ([Stephen *et al.*, 1978](#_ENREF_212)), twenty-tow out of 428 (5.1%) (32), nine out of 100 (9%) (33), fourteen out of 200 (7%) (34), fourteen out of 170 (8.2%) (35), 13 out of 94 (13.8%) (36) and seven out of 100 (7%) . These reports highlight the importance of the pathogen as a causative agent for spontaneous human abortions in this ecozone of the world.

25 out of 120 (21%) (37) have a higher occurrence compared with a present study.

However, *L. monocytogenes* was recovered from meat and ready to eat food according to studies carried out in Sudan, isolated 204 out of 500 (40%) from Fresh Raw Dressed Broiler Chicken, and isolated 3% of *L. monocytogenes* from Ready to Eat Vended Food of Meat Origin (38), this was indicted to infection of pregnant women with *Liseria* from consumption of contaminated food.

In other study in carried out in Sudan, isolated the *Listeria* from blood in case of Puerperal Sepsis (39).

The present study shows the most effective age (31- 36 years) this agreed with . This may be attributed to increase marriage and sexual activity with pregnancy at this age group who increased consumption of milk, milk products, fruits and vegetables infected with *Listeria* spp.

The majority of positive cases of *L. monocytogenes* 4 (80%) were recovered from women having single abortion during their marriage period, this agreed with (40).

Most of the positive samples infected with *L. monocytogenes* 3 (60%) were obtained from the vaginal swab, this agreed with (41).

There is a lack of data for low-income countries and developing countries, the studies only find data from high income and middle-income regions, and said certain assumptions had to be made to produce global

**5- Conclusion**

From this study we can conclude that, *L. monocytogenes* has on association with the spontaneous miscarriage in Sudanese pregnant women.

Also we found different percentage of prevalence between the vaginal and high vaginal swab samples, and the most cases of listeriosis found in second trimester gestational age of pregnant women at sampling.

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