Study of bacteria *Listeria monocytogenes* in spontaneous aborted women in Salah Al-deen province

Alaa Zanzal Ra'ad Al-dorri
Microbiological Department, College of Medicine ,Tikrit University, Tikrit , Iraq
E-mail: alaa.zanzal@yahoo.com

Abstract:
This study was performed between January 2013 to the end of June 2013, involving 94 spontaneous aborted women at 15-45 years who attended TGH in Salah Al-deen province. They were examined and taking placental pits and cervical swabs. For diagnosis, used Gram staining, cultures, biochemical tests, API system and molecular technique including polymerase chain reaction (PCR).
The spontaneous aborted women with *Listeria* infection was 13 (13.82%) out of 94 aborted women, while 81(81.86%) of aborted women had other causes of abortion. The most affected age group with *Listeria* infection was 25 – 34 years old represented 7 (53.84%) aborted women. The most infected patients from rural area was 61.53%, while in urban was 38.46%. The most aborted women with *Listeria* infection were had previous abortions within first trimester (less than 12 weeks of gestational age) were 8 (100%) cases. The pathogens isolated from placenta pits and cervical swabs was *Listeria monocytogenes* represented 11(37.93%) out of 29 isolated pathogens, while *Listeria ivanovii* was 2 (6.80%). PCR was used for detection of virulence associated gene of *Listeria monocytogenes* including *hly A* which had been found to be amplifying of their DNA fragments. The PCR products showed the eleven isolates of *Listeria monocytogenes* had the *hly A* gene.

Key words: *Listeria monocytogenes*, abortion, Listeriosis.

Introduction:

Listeriosis is one of the most important food-borne diseases of humans. The disease can induce septicemia, meningitis and encephalitis. *Listeria monocytogenes* has also been associated with gastroenteritis with fever and it cause severe invasive disease in human. In pregnant women, intrauterine or cervical infections may result in spontaneous abortion or stillbirths, and may be preceded by influenza-like signs, including fever [1].
Listeria monocytogenes is mostly responsible for human infection but occasionally infection with *L. ivanovii* and *L. seeligeri* has been reported. Nyfeldt reported the first human case of listeriosis in 1929. Incidence of human listeriosis varies between countries ranging from 4.4 to 7.4 per million of the population annually. All *L. monocytogenes* strains have similar pathogenicity, regardless of geographic origin. Although debilitated immune status due to, diabetes mellitus, pregnancy, cardiovascular disease, neoplastic disease, and hemodialysis failure are important factors in the pathogenesis of listeriosis, other factors also play important role such as genetic, behavior and age [2].
Several outbreaks of listeriosis in human associated with the consumption of milk and dairy products have occurred since 1980, and the mortality rate about 30% of these outbreaks [3]. *Listeria monocytogenes* may directly contaminate milk. There was rising in the numbers of human cases in several countries (including the UK) together with evidence for food borne transmission of this disease has been much renewed interest [4].
In 2000, infections caused by *Listeria monocytogenes* were reported in 7.4 per million pregnant women, accounting for about 30% of all cases. Overall, pregnant women are more susceptible to acquiring listeriosis, and the risk of listeriosis in pregnant women has been reported to be 17 times that of the normal population [5, 6, 7]. The frequency is as high as 50% of neonate. Although listeriosis is known increasingly as a serious threat the health, there is little study in some countries, especially Asian countries [8].
L. monocytogenes has a saprophytic life and occurs widely in nature. A variety of animals including domestic farm animals can carry the bacterium, also it can survive for long periods in a plant–soil environment, and in a marine environment as it is a salt tolerant organism. It is transmitted to human by consuming food contaminated with this pathogen such as milk and dairy products, meat, poultry, vegetables, salads and seafood [9]. Moreover, person-to-person transmission of *L. monocytogenes* was reported to be possible through sexual contact as the bacterium was isolated from human semen and isolated from urethral swabs from males who patronized prostitutes in Zaria, northwestern Nigeria. This establishes the possibility of a pregnant mother being infected with *L. monocytogenes* through sexual intercourse, which could lead to spontaneous abortion [10].
L. monocytogenes is a Gram-positive, rod bacilli non-spore forming, facultative intracellular exquisitely adaptable environmental bacterium with a powerful array of regulated virulence factors[11]. *L. monocytogenes* can grow at temperatures below 4^{O}C, and can tolerate high salt environment and a pH between 5.4–9.6. *L.monocytogenes* strains are serotyped according to variation in the somatic (O) and flagellar (H) antigens [12].
The aim of the study is: To determine the occurrence and distribution of *Listeria monocytogenes* in

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

spontaneous aborted women in Salah Al-deen province.

Materials and Methods:

The study population were 94 spontaneous aborted women, at aged between 15-45 years old, who were attending the outpatient of gynecological and obstetric clinic in Tikrit Teaching Hospital in Tikrit province in the period from first of January 2013 to the end of June 2013. Most of the patients were suffering from spontaneous abortion. The samples were comprised of placental pits and cervical swabs were cultured on selective media called *Listeria* agar media (Oxoid-England) for growth *Listeria spp.*, and streaked the blood agar, incubated aerobically at 35-37 ^{O}C/24-48 hour, which showed small, circular, smooth edge colonies, □-hemolytic on blood agar, and brown to black hallow of aesculin hydrolysis around the colonies on *Listeria* selective media. Then subsequent sub-culture on *Listeria* selective agar. The suspected colonies were confirmed and further identified by standard identification and biochemical tests including Gram stain microscopy, catalase test was positive, oxidase test, methyl red, Voges - Proskauer (MR-VP) reactions, fermentation of sugars (glucose, xylose, rhamnose, and mannitol), citrate utilization, urease production test, and indole test. Tumbling motility at 20-25^{O}C (at room temperature), in addition to API system [13] as in picture No. 1, 2 and 3.

DNA extraction from *Listeria monocytogenes* isolates:

EZ-10 Spin Column Genomic and Minipreps (®BIO BASIC INC.) kit will isolate all genomic DNA. By taking the advantage of silica-based DNA purification technology, DNA is selectively adsorbed in silica-based membrane embedded in EZ-10 Spin Column. Other components and impurities flow through the column or are washed away during wash steps.

The purification procedure using in these kits do not require use of hazardous compound such as phenol, chloroform, or CsCl. DNA is purified without additional steps of ethanol precipitation.

The reaction size is 50 µl, consist of 1 Ml (10 pmol) forward and 1 Ml (10pmol) reverse primers. The cycling conditions for PCR included an initial denaturation of DNA at 95 ^{o}C for 2 min followed by 35 cycles each of 15 s denaturation at 95 ^{o}C, 30 s annealing at 60 ^{o}C and 1 min 30 s extension at 72 ^{o}C, followed by a final extension of 10 min at 72^{o}C and held at 4 ^{o}C. The used primer for virulence-associated gene was amplified under the similar PCR conditions and amplification cycles [11]. The result of PCR product was analysed by agarose gel electrophoresis (1.5% agarose), stained with ethidium bromide (5mg/ml) was fluorescent dye that intercalates DNA and excited viewing the fluorescence by UV.

The standardized PCR protocol for 50 µl reaction mixture. The primer for detection of hemolysin gene (*hly*A), of *L. monocytogenes* used in this study was (*hly*A) forward 5`GCAGTTGCAAGCGCTTGGAGTGAA 3`, and reverse 5`GCAACGTATCCTCCAGAGT GATCG 3`. The size band at (456 bp) according to Paziak-Domanska et al [14].

Results:

Figure (1) showed the aborted women with positive infection of Listeria in placental pits and cervical swabs, represented 13 (13.82%) pregnant women out of 94 aborted women, while 81 (86.17%) aborted women had another causes of abortion other than Listeria infection.

Table (1) showed the distribution of aborted pregnant women with *Listeria* infection and without its according to the age, the most affected age group with Listeria infection was 25 – 34 years old who represented 7 (53.84%) aborted women, while the age group was 15 – 24 years old represented 4 (30.76%) aborted women, but the less affected age group was 35 – 44 years old represented 2 (15.38%).

Table (2) showed the distribution of aborted women with *Listeria* infection according to the residence. The most pregnant women who had abortion with and without *Listeria* infection were from rural area represented 61.53% and 70.37% respectively, while in urban were 38.46% and 29.62% respectively.

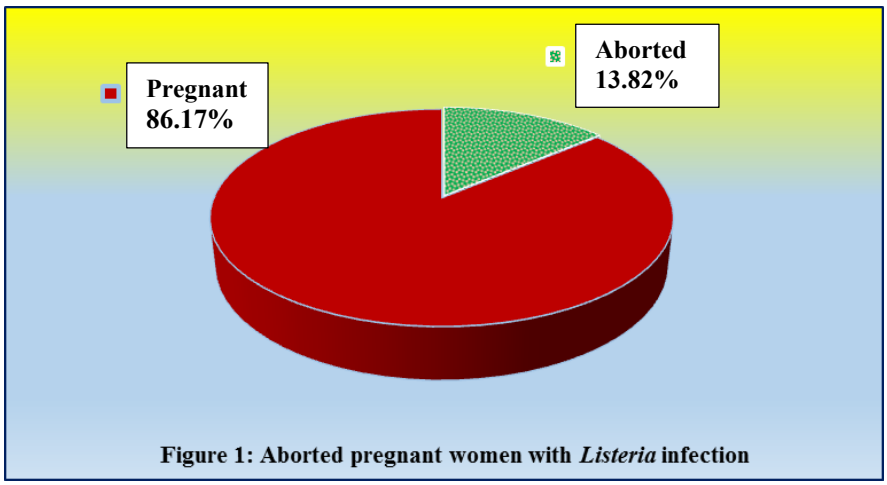


Figure 1: Aborted pregnant women with *Listeria* infection

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

Table 1: Distribution of aborted pregnant women with *Listeria* infection according to age.

Age group (yr.)	*Listeria* + ve		*Listeria* - ve		Total
	No.	%	No.	%	
15 – 24	4	30.76	28	34.56	32
25 – 34	7	53.84	38	46.91	45
35 – 44	2	15.38	15	18.51	17
Total	13	100%	81	100%	94

Table 2: Distribution of aborted pregnant women with *Listeria* infection according to residence

Residence	*Listeria* + ve		*Listeria* - ve		Total
	No.	%	No.	%	
Urban	5	38.46	24	29.62	29
Rural	8	61.53	57	70.37	65
Total	13	100%	81	100%	94

Table (3) showed the frequency of pregnant women with recurrent abortion previously. The most aborted women with *Listeria* infection were had past abortions within first trimester (less than 12 weeks of gestational age) were 8 (100%) cases out of 8 abortions, while they had not in second and third trimester. Other aborted women without *Listeria* infection had past abortions in first trimester were 22 (68.75%) cases out of 32 abortions, while in second and third trimester were 5 (15.62%) cases for each one.

Table 3: Frequency of pregnant women with history of abortion

Gestational age	*Listeria* + ve		*Listeria* - ve		Total
	No.	%	No.	%	
First trimester	8	100	22	68.75	30
Second trimester	0	0	5	15.62	5
Third trimester	0	0	5	15.62	5
Total	8	100%	32	100%	40

Figure (2) showed aborted women with *Listeria* infection, 13 aborted pregnant women with *Listeria spp*. infection out of 94 cases. There would be 11/13 aborted women with *Listeria monocytogenes*, while 2/13 cases with *Listeria ivanovii*.

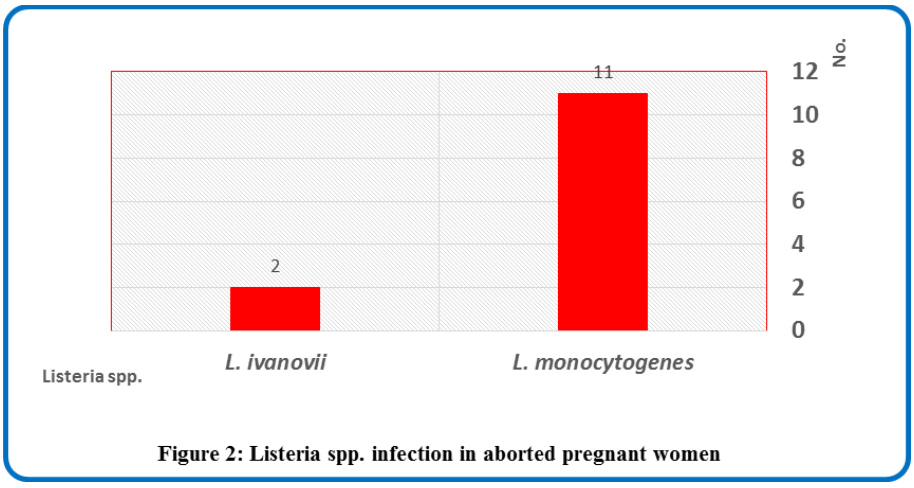
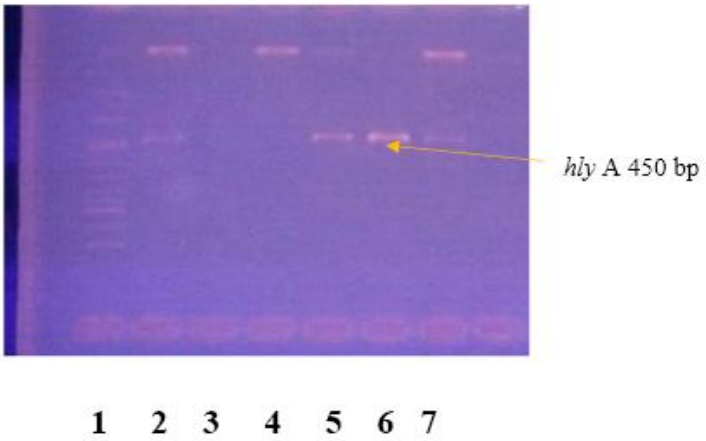


Figure 2: Listeria spp. infection in aborted pregnant women

Picture (1) showed the PCR standardized for the detection of virulence associated gene of *Listeria monocytogenes* had been found to be capable of amplifying the gene in the form of their DNA fragments. These findings commensurate with the published work for detection of *hlyA* gene [14].



Picture 1: Agarose gel electrophoresis of amplified PCR products for diagnosis of *Listeria monocytogenes*, using hemolysine primers gene. Lane 1 marker size 100-1000 bp, (*hly* A) gene in lane 5 and 6 band at 450 bp.

Discussion:

Listeria species are found in soil, water, a large variety of foods, and the feces of humans and animals. However, only *Listeria monocytogenes* and, rarely, *L. ivanovii* are pathogenic for humans, out of ten known *Listeria* species. Healthy animals may harbour pathogenic *Listeria* in their gastrointestinal tracts. Vegetables may become contaminated through soil or manure used as fertilizer. Food may occasionally contain *L. monocytogenes* include a wide variety of ready to-eat or raw foods, such as raw milk or meat and their products, raw mushrooms, soft cheese and seafood. Foods contaminated with *Listeria* look, smell, and taste normal.

Pregnant women have 17-fold increased risk of developing *listeria* bacteraemia, two-third of babies born to such mothers develop clinical listeriosis. The source of infection is generally from animals through undercooked meat or chicken [15]. Neonates acquire infection by transplacental spread, aspiration of infected meconium or in rare instances of cross-contamination in newborn nurseries. Even with appropriate treatment, the overall mortality with listeriosis is 30% [16].

In the present study shows 13(13.82%) aborted pregnant women with *Listeria monocytogenes* infection, while 81(86.17%) aborted pregnant women with other causes. This agreed with [17], who reported 14(8.04%) pregnant women with spontaneous abortion with *L. monocytogenes* infection. Also its agreed with research from Iran that reported 25 pregnant women had abortion with *L. monoytogenes* infection from 120 pregnant women [8] and others mentioned 7 pregnant women from 96 had abortion due to *L. monocytogenes* [18], in addition to that in Israel from 1995 to 1999, 161 cases were identified 70 (43%) were perinatal infections with a fetal mortality rate 45% [19]. Other authors [11] in India reported the occurrence of pathogenic *L. monocytogenes* in cases of spontaneous abortions was 3.3%. The WHO [20] mentioned in developing countries the proportion of perinatal cases (the period close to or soon after birth) was 20.7%. Other study [21] mentioned the pregnant women are about 20 times more likely to get listeriosis than other healthy adults. It can result in miscarriage or stillbirth. Newborns may also have low birth weight, septicemia and meningitis.

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 estimations. This assumption could not be checked against observed data and so may greatly affect the results. The reason of the mother is more susceptible that her immune system is compromised in addition to that the bacteria got into her placenta.

The present study shows the most effective age (25-34 years) of aborted pregnant women with Listeria infection was 7(53.84%) then (15-24 years) and (35-44 years) were 4(30.76%) and 2(15.38%) respectively especially from rural area 8(61.53%). This agreed with [22] mentioned the average age of participants with *Listeria* infection was 25.6 $\pm$ 7.6 years in the case group, also agreed with [17] that reported most patient with spontaneous abortion had *Listeria* infection at age of (30-34 years). 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*.

Spontaneous abortion and miscarriage mean the termination of pregnancy between 20 weeks gestation and the delivery of a fetus weighing less than 500 g [23]. The present study shows aborted pregnant women with *Listeria* infection most common in 1st trimester (<12 wk) was 8 (100%). This was agreed with [22] that reported pregnant women with abortion associated with *Listeria* infection at gestational age of early (< 12 wk) was 54(60.68%) and at late (12–20 wk) was 35(39.32%).

The pathogenic *Listeria spp*. should be based on virulence factors. The virulence cluster of *Listeria spp*., comprises six coherent chromosomal genes encoding haemolysin (listeriolysin-O) encoded by *hly* A, a phosphatidylinositol phospholipase C encoded by *plc* A, a phosphatidylcholine phospholipase C encoded by *plc* B, a metalloprotease and a surface actin polymerization protein actA, all physically linked in a 9-kb chromosomal island referred to as *Listeria* pathogenicity island 1 (LIPI-1) [24]. Only *Listeria monocytogenes* and *Listeria ivanovii* are generally known to be pathogenic. *Listeria monocytogenes* is known to be pathogenic to both humans and animals, while *Listeria ivanovii* is generally regarded as pathogenic, only to animals. However, there are reported sporadic cases of human infections with *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua* and *Listeria welchimeri* [25].

The PCR standardized for the detection of virulence associated gene of *L. monocytogenes* has been found to be capable of amplifying all the genes in the form of their DNA fragments under the same amplification and cycling conditions. These findings commensurate with the published work for detection of *hly* A gene [14]. In the present study, the expression of hemolytic activity were presented by all the eleven isolates of *L. monocytogenes*.

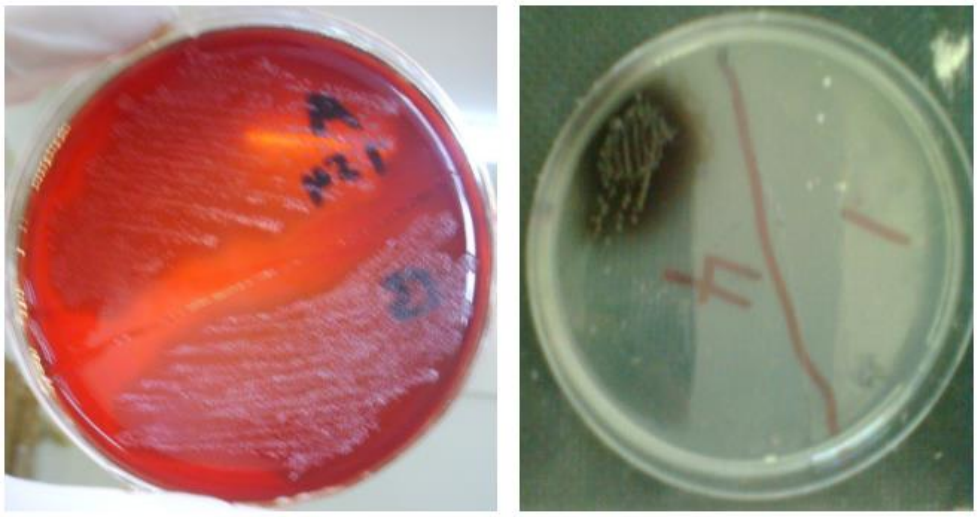
The results show that PCR is a more sensitive, easier and faster method in comparison to culture for detecting *L. monocytogenes*. It is obvious that quick diagnostic and starting antimicrobial therapy at the right time can prevent and decrease abortion`s complications, so it is suggested that using PCR in detecting *L. monocytogenes* can be more effective.

Conclusion:

Generally, the notable reports or researches about listeriosis and its sequence complications such as human abortions in Iraq was little. We can conclude that one of the most important causes of spontaneous

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

abortion in human is *Listeria* infection especially *L. monocytogenes* in rural area and PCR is more sensitive and specific tool.



A B

Picture 2: A; *L.monocytogenes* with β-hemolysis on blood agar, B; *L.monocytogenes* on selective *Listeria* agar with black hallow around colonies.

References

1. NB: Version adopted by the World Assembly of Delegates of the OIE in May 2014 OIE Terrestrial Manual, Chapter 2. 9. 7. *Listeria monocytogenes*.
2. Vishal Singh Parihar. (2004) Zoonotic Aspects of *Listeria monocytogenes* with Special Reference to Bacteriology. Master of Science Programme in Veterinary Medicine for International Students Faculty of Veterinary Medicine and Animal Science. Swedish University of Agricultural Sciences, Uppsala.
3. Griffiths MW. (1989) *Listeria monocytogenes* its importance in the dairy industry. J Sci Food Agric. 47:133-158.
4. B. A. Abbas and G. M. Jaber. (2012) Occurrence of *Listeria monocytogens* in raw milk of ruminants in Basrah province. Iraqi Journal of Veterinary Sciences; Vol. 26, No. 1: (47-51).
5. Gellin BG, Broome CV, Bibb WF, Weaver RE, Gaventa S, and Mascola L. (1986) The epidemiology of listeriosis in the United States. Listeriosis Study Group. Am J Epidemiol 1991; 133: 392–401.
6. Ciesielski CA, Hightower AW, Parsons SK, and Broome CV. (1988) Listeriosis in the United States: 1980–1982. Arch Intern Med; 148: 1416–9.
7. Mylonakis E, Paliou M, Hohmann EL, Calderwood SB, and Wing EJ. (2002) Listeriosis during pregnancy: a case series and review of 222 cases. Medicine (Baltimore); 81:260–9.
8. Ali Haghroata, Ahmad F. Shakh, Mohsen M. and Shoosntari. (2015). Investigation on the seroprevalence of *Listeria monocytogenes* on women with spontaneous abortion. Comp Clin Pathol; 24: 153- 156.
9. Mostafa A. El-Shenawy, and Mohamed A. El-Shenawy. (2006) *Listeria spp.* in the coastal environment of the Aqaba Gulf, Suez Gulf and the Red Sea. Epidemiol. Infect.; 134, 752–757.
10. Onyemelukwue, G.C., Lawande, R.V., Egler, L.J. and Mohammed, I. (1983) *Listeria monocytogenes* in northern Nigeria. J Infect; 6: 141 – 145.
11. S. Kaur, S.V.S. Malik, V.M. Vaidya and S.B. Barbuddhe. (2007) *Listeria monocytogenes* in spontaneous abortions in humans and its detection by multiplex PCR. Journal of Applied Microbiology 103: 1889–1896.
12. S. Kalekar and et al. (2011). Genotypic characterization of *Listeria monocytogenes* isolated from humans in India. Annals of Tropical Medicine & Parasitology; Vol. 105, No. 5, 351–358.
13. Rahimi E, and et al. (2012) Prevalence and Antimicrobial Resistance of *Listeria species* isolated from traditional dairy products in Chahar, Mahal and Bakhtiyari, Iran. Bulgarian Journal of Veterinary Medicine; 15: 115-122.
14. Paziak-Domanska, B., Bogulawska, E., Wiekowska-Szakiel, M., Kotlowski, R., Rozalska, B., Chmiela, M., Kur, J., Dabrowski, W., et al. (1999) Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of Listeria monocytogenes in meat foods. FEMS Microbiol Lett 171, 209–214.
15. Bortolussi R. (1990) Neonatal listeriosis. Semin Perinatol; 14:44-8.
16. Mitchell RG. (1996) *Listeria*, erysipelothrix. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney practical medical

microbiology. 14 th ed. Edinburgh: Churchill Livingstone: 309-15.
17. Shindang, J., Shindang, C.O. and Ekwempu, A.I. (2013) Incidence of *Listeria monocytogenes* and Other Bacteria in Spontaneous Abortion Cases in Jos. Nig J. Biotech.; Vol. 25: 18 – 22.
18. Smith, B., Kemp, M., Ethelkeng, S., Schiellerup, P., Bruun, B, G., Gerner-Smidt, P. and Chritensen, J. J. (2009) *Listeria monocytogenes*: maternal-fetal infections in Denmark 1994 – 2005.Scand J. Infect. Dis.,; 41: 21 – 25.
19. Mirdamadi, S. (2005) Immunological and microbiological study of abortion samples for serotype determination of *Listeria monocytogenes* in Iran. ESCMID; 24: 1245 – 1345.
20. Rachel Arthur. (2014) Urgent efforts needed to fill *Listeria* data gap in developing countries: WHO study. Last updated on 06-October.
21. WHO, Regional Office for South-East Asia. Basic Information on Emerging Infectious Diseases (EIDs): *Listeriosis:* What we should know
22. Mahin Jamshidi, Abdolreza Sotoodeh Jahromi, Parivash Davoodian, Malihe Amirian, Mehrangiz Zangeneh, and Fatemeh Jadcareh. (2009) Seropositivity for *Listeria monocytogenes* in women with spontaneous abortion: A case-control study in Iran. Taiwan J Obstet Gynecol March; Vol 48, No 1.
23. Shiers, C. U. (2001) Myles Textbook for Midwives. 13th Ed. London. Churchil Living Stone Company; pp.245 – 235.
24. Vazquez-Boland, and et al. (2001) *Listeria* pathogenesis and molecular virulence determinants. Clin Microbiol Rev; 14, 584–640.
25. Perrin, M. Bemer M. and Delamare, C. (2003) Fatal case of *Listeria innocua* Bacteremia. Journal of Chemical Microbiology; 41:5308 – 5309.

دراسة بكتيريا *Listeria monocytogenes* في النساء المجهضات تلقائيا في محافظة صلاح الدين

آلاء زنزل رعد الدوري

فرع الأحياء المجهرية ، كلية الطب ، جامعة تكريت ، تكريت ، العراق

الملخص

أنجزت هذه الدراسة خلال الفترة ما بين كانون الثاني 2013 الى نهاية حزيران 2013 متضمنة 94 امرأة مجهضة تلقائيا بين أعمار 15 – 45 سنة اللواتي راجعن مستشفى تكريت التعليمي في محافظة صلاح الدين وتم فحصهن وأخذ مسحات من حفر المشيمة وعنق الرحم وشخصت باستخدام صبغة الكرام والزرع والفحوصات الكيموحيوية ونظام API والتقنية الجزيئية باستخدام تفاعل السلسلة المتبلمرة.

نسبة النساء المجهضات تلقائيا والمصابات ببكتريا *Listeria* كانت (13.82%) 13 من 94 امرأة مجهضة بينما كانت نسبة (81.86%)81 من النساء المجهضات تلقائيا يمتلكن مسببات أخرى. معظم المجاميع العمرية المصابة كانت بين 25 – 34 سنة بنسبة (53.84%) 7 امرأة مجهضة. ومعظم المرضى من المناطق الريفية بنسبة 61.53% بينما المناطق الحضرية كانت 38.46%. وكذلك معظم النساء المجهضات والمصابات ببكتيريا *Listeria* أمتلكن اجهاضات مسبقة خلال الثلث الأول بنسبة (100%) 8. العزلات من المسحات المأخوذة من حفر المشيمة وعنق الرحم كانت بكتريا *Listeria monocytogenes* بنسبة (37.93%)11 من مجموع 29 عزلة *Listeria* بينما كانت *Listeria ivanovii* بنسبة 2 *(6.80%)* . استخدمت تقنية تفاعل السلسلة المتبلمرة للكشف عن جين عامل الضراوة المسبب للتحلل الدموي *hly A* وقد أظهرت نتائج تفاعل السلسلة المتبلمرة أن الأحد عشر عزلة من *Listeria monocytogenes* كانت تحمل جين *hly A* .