

Full Length Research Paper

Prevalence and antimicrobial resistance profiles of *Listeria monocytogenes* in spontaneous abortions in humans

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Accepted 09 February, 2019

Listeria monocytogenes is a causative agent of listeriosis. Clinical manifestation of invasive listeriosis is usually severe and include sepsis and meningoencephalitis. Uterian infection in listeriosis depend on gestational age varies from preterm labour, delivery of infected neonate to abortion. The present study investigated the incidence and antibiograms of the organism in clinical samples were collected from women with spontaneous abortions in Iran. Clinical samples were collected from patients with spontaneous abortions hospitalized in Shariati hospital in Tehran during December 2009 and November 2010. Antibiogram was done by disk-agar method. *L. monocytogenes* was isolated from 9 of 100 samples. Out of 9 isolates of *L. monocytogenes* 3 (16.66%), 0 (0%), 1 (4%), 3 (12%) and 2 (8%) were isolated from placental tissue, blood, urine, vaginal and rectal swabs respectively. Resistance to Penicillin G had the highest rate. Because of high incidence of *L. monocytogenes* in this study, in women with history of abortions or bad obstetric history, diagnosis procedures for detection of *L. monocytogenes* and on time treatment is recommended additionally because of high resistance rate of bacteria to Penicillin G in this study, antibacterial susceptibility before initiation of treatment is recommended.

Key words: *Listeria monocytogenes*, spontaneous abortion, antibiotic resistance.

INTRODUCTION

Listeria monocytogenes is a Gram positive, non sporeforming, facultative intracellular and adaptable environmental bacterium. Although most bacteria do not grow or grow weakly at temperatures below 4°C, *L. monocytogenes* survive in temperatures range use for refrigeration. As a result, *L. monocytogenes* is an important food born pathogen in ready-to-eat foods that have been refrigerated (Ramaswamy et al., 2007; Salyers and Whitt, 2002).

This bacterium is the cause of listeriosis. Listeriosis is an infection with *L. monocytogenes* that pregnant women, neonates, elderly and immunocompromised persons are at high risk of infection. Although listeriosis is uncommon, fatal rate in high risks can be as high as 30% (Ramaswamy et al., 2007). Infection of pregnant women is important because of two reasons. First, placenta is a suitable niche for *L. monocytogenes* growth. Prevalence of listeriosis in general population is 0.7 in 100000 but this prevalence is 12 in 100000 in pregnant women (that is, a 17-fold increase) (Kaur et al., 2007).

Clinical manifestation of invasive listeriosis is usually severe and include sepsis and meningoencephalitis.

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Uterian infection in listeriosis depend on gestational age varies from preterm labour, delivery of infected neonate to abortion. Latent listeriosis in pregnant women lead to habitual abortion, intrauterine death and malformation of fetus. High fatality rate of listriosis, serious outcomes for pregnancy and transmission through the consumption of refrigerated contaminated foods makes suitable antibiotic therapy extremely important (Vela et al., 2001). Although most isolates of these bacteria are susceptible to the most antibiotics of choice, resistance also have been reported in food, environmental and clinical isolates (Morvan et al., 2010). Increasing reported resistance cases, indicate the necessity of monitoring the antimicrobial susceptibility of *L. monocytogenes* (Vela et al., 2001).

The first multidrug resistant strain was reported in France in 1988 (Poyart salmeron et al., 1990). Thereafter, resistant strains to one or more antibiotics also have been reported (Charpentier and Courvalin, 1999). So, the aim of this study was to detect *L. monocytogenes* in pregnant women to prevent serious side effects of listeriosis in them, especially in neonates and to determine antibiotic susceptibility of strains for effective treatment of pregnant women and neonates.

MATERIALS AND METHODS

In this study, a total of 100 samples including blood, urine, placental tissue, fecal and vaginal swabs were collected from patients with spontaneous abortions hospitalized in Shariati hospital, Iran, during December 2009 and November 2010.

Enrichment, culturing, morphological and biochemical identification

5 ml from each of blood and urine samples and 25 g of the placental tissue were inoculated in to 50 and 225 ml of TSBYE (Tryptic soy broth plus 0.6% yeast extract, Merck, Germany). Each of fecal and vaginal swabs was inoculated into 10 ml of TSBYE. All of samples were incubated at 4°C. After one or two weeks until 6 months incubation, aliquots from enrichment broth (TSBYE), were streaked onto PALKAM Agar (Merck, Germany) and listeria selective agar (Himedia, India) and plates were incubated at 35°C for 24 to 48 h. The green shiny colonies with diffuse black shadow around them on PALKAM agar because of hydrolysis of esculin and yellow small colonies on listeria selective agar were suspected to be *Listeria*. About 5 typical presumptive *Listeria* colonies from each culture media were purified on Brain Heart Infusion agar (Merck, Germany) and identified using morphological, cultural and biochemical tests. Confirmation of isolates was done by Gram's staining, catalase reaction, oxidase test, methyl red, voges proskauer (MR-VP) tests and fermentation of sugars (xylose, rhamnose, mannitol and -methyl D-mannopyranoside). All the biochemically characterized isolates were tested for haemolysis on Sheep Blood Agar and Christie, Atkins, Munch Petersen (CAMP) test. The confirmed isolates as *L. monocytogenes* were stored at maintenance medium including pepton (1%) and glycerol (15%).

Antibiotic susceptibility testing

1 ml of bacterial suspension was transferred onto Mueller- Hinton

Agar and spread in all directions on plate. The turbidity of broth after incubation was adjusted with sterile saline to obtain turbidity comparable of 0.5 McFarland standard. The susceptibility of isolates were tested by the disk agar method as standardized by the national for clinical laboratory standards (NCCLS, 1998). The following antibiotics were used: chloramphenicol (10 µg), tetracycline (25 µg), penicillin G (10U), streptomycin (10 µg), trimethoprim (5 µg), ciprofloxacin (5 µg), ampicillin (10 µg), cephotoxim (30 µg), norfloxacin (10 µg), erythromycin (15 µg) (Himedia, India). *L. monocytogenes* ATCC 7644 was used as the reference strain.

RESULTS

L. monocytogenes was found in 9 samples (9%) studied. Table 1 shows that, the bacterium was isolated from vaginal swab and placental bit with highest frequency (3% for each of them), but *L. monocytogenes* was not isolated from blood samples.

Antimicrobial susceptibility testing of *L. monocytogenes*

Of 9 confirmed *L. monocytogenes* isolates, 7 (77.77%) were found to be resistant to one or more antibiotics. Resistance to penicillin G, cephotaxim, chloramphenicol and streptomycin were in 77.77, 77.77, 11.11 and 11.11% of strains respectively. Antibiotic resistance was not found for tetracyclin, trimethoprim, ciprofloxacin, norfloxacin, ampicillin and erythromycin (Table 2). Four different resistance patterns were observed (Table 3).

DISCUSSION

About one-third of reported human listeriosis cases is reported during pregnancy, which may result in spontaneous abortion (CDC, 2005). Reports of listeriosis from humans in Iran are uncertain, either because of failure to identify the isolate, its rarity, improper isolation techniques or lack of awareness. In the present study, of 100 samples collected from women with spontaneous abortions, 9 isolates were identified as *L. monocytogenes*. Contamination rate of samples in women with spontaneous abortion was 2, 3, 3, 1 and 0% for rectal swab, vaginal swab, placental bit, urine and blood respectively.

Different incidence rates of *L. monocytogenes* has been reported from several countries. The incidence rate of *L. monocytogenes* in this study (9%) was higher than the earlier reports on the isolation of *L. monocytogenes* from three of 100 (Bhujwala et al., 1973), four of 305 (Kaur et al., 2007) and one of 958 (Stepanovich et al., 2007). The difference reported among the studies can be due to differences in the population under study include race, culture, geographical region, nutrition and laboratorial diagnosis methods.

Table 1. Incidence of *L. monocytogenes* in various samples from spontaneous abortion.

Various samples	No. of positive isolates	% of positive isolates
Rectal swab	2	2
Vaginal swab	3	3
Placental bit	3	3
Urine	1	1
Blood	0	0

Table 2. Susceptibility of *L. monocytogenes* to 10 antimicrobial agents.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Chloramphenicol	22.23	66.66	11.11
Penicillin G	-	22.23	77.77
Streptomycin	88.89	-	11.11
Tetracycline	88.89	11.11	-
Trimethopirim	100	-	-
Ciprofloxacin	66.67	33.33	-
Ampicillin	88.89	11.11	-
Cephotaxim	22.23	-	77.77
Norfloxacin	100	-	-
Erythromycin	100	-	-

Table 3. Resistance patterns of *L. monocytogenes* isolated from clinical samples of women with spontaneous abortion.

Samples	No. of isolates	Resistance pattern	No. of resistant strains	Resistant (%)
Rectal swab	2	CTX, P	2	100
Vaginal swab	3	CTX, P, C, S	1	33.33
		CTX, P	2	66.67
Placental bit	3	CTX, P	1	33.33
		CTX	1	33.33
		P	1	33.33
Blood	-	-	-	-
Urine	1	-	-	-

Also, the result of this study is in agreement with the earlier reports on the isolation of *L. monocytogenes* from seven of 100 (Shayan et al. unpublished) and twenty-two of 428 woman with a bad obstetric history (Kargar and Ghasemi, 2009), highlighting the role of *L. monocytogenes* as a causative agent of human abortions. Three isolates of *L. monocytogenes* was recovered from placental tissues of women with spontaneous abortion. Two of these three women had previous bad obstetric history. Infection with *L. monocytogenes* in pregnant women sometimes giving rise to urinary tract infection symptoms (Benshushan et

al., 2002). In present study, *L. monocytogenes* was isolated from the urine of a woman with spontaneous abortion.

In our study *L. monocytogenes* was detected in fecal and vaginal swabs of patients with spontaneous abortion. In two cases (22.22%), vaginal and rectal swabs were belong to same patient and in one case (11.11%) *L. monocytogenes* was detected from placental tissue, rectal and vaginal swabs of same patient. These findings highlights the role of *L. monocytogenes* at spontaneous abortions in these women.

In the present study, out of 9 isolates of *L.*

monocytogenes 3 (16.66%), 0 (0%), 1(4%), 3 (12%) and 2 (8%) were isolated from placental tissue, blood, urine, vaginal and rectal swabs respectively. In a study of fecal, cervicovaginal and oropharyngeal carriers, Lamont and Postlethwaite (1986) were detected *Listeria* species in 54 healthy pregnant women and 60 healthy non-pregnant women in Scotland. *L. monocytogenes* was isolated from a pregnant women (2%) and to healthy non pregnant women (3.4%) fecal samples but organism was not detected from cervicovaginal and oropharyngeal samples (Lamont et al., 1986).

Until recently, *L. monocytogenes* was thought to be susceptible to antibiotics used for listeriosis treatment including penicillin combined with aminoglycosides. However, many resistant strains have been detected in food and various cases of listeriosis in recent years (Charpentier et al., 1995). In our study, *L. monocytogenes* was highly sensitive to trimethoprim, erythromycin, norfloxacin (100%), sensitive to streptomycin (88.89%), chloramphenicol (88%), tetracycline (88.89%), ciprofloxacin (66.67%), ampicillin (88.89%), but resistant to penicillin G (77.77%) and cefotaxim (77.77%). In agreement with our study about resistance to penicillin, Pozark and Murano (2002) reported one isolate of *L. monocytogenes* that was susceptible to all antimicrobial agents except penicillin. They also reported penicillin and gentamicin resistant strain of *L. monocytogenes* that provide evidence of emergence of multiresistant *L. monocytogenes* strains, pointing to an increase in the potential threat to human health posed by this pathogen (Prazak et al., 2002). Susceptibility to chloramphenicol (88.89%) and resistance to cefotaxime (77.77%) was in agreement with Safdar and Armstrong (2003) study. Over all there were high diversity of antibiogram profiles among studies that tested antibiotic resistance in clinical isolates of *L. monocytogenes*.

In contrast to the present study, Morvan et al. (2010) found no strain that was resistant to penicillin in France. High incidence of *L. monocytogenes* in patients with spontaneous abortion in this study and resistance to penicillin highlights importance of pregnant women education about consumption of ready to eat foods and hygienic system and young physicians about asymptomatic or mild flu-like symptoms of listeriosis in pregnant women. At the aspect of penicillin resistance, because penicillin plus gentamicin is drug of choice for listeriosis treatment in most hospitals in Iran and other countries, antibiogram for on time and accurate treatment to prevent risks that threat pregnant women and their fetuses is essential.

ACKNOWLEDGEMENTS

This study was supported financially and sponsored by Cell and Molecular Biology Research Center and Microbiology Group of Tehran Medicine University.

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