

Incidence of *Listeria monocytogenes* and Other Bacteria in Spontaneous Abortion Cases in Jos.

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Abstract

The involvement of *Listeria monocytogenes* in spontaneous abortion in Jos was investigated. One hundred blood and 100 placenta samples were collected from spontaneous abortion patients in Jos. The samples were inoculated first into *Listeria* enrichment broth. Incubation was at 37° C, followed by cold enrichment at 0° C for samples that did not yield any growth at 37° C. Further sub-inoculations were made on *Listeria* selective agar. In addition, parallel inoculations were also made on blood, chocolate and MacConkey agar media using standard bacteriological methods. *Listeria*-like organisms were subjected to biochemical and serological investigations using type 1a, 4b and polyvalent sera supplied by Difco laboratories (Detroit, Michigan). *Enterococcus faecalis*, *Proteus mirabilis*, *Escherichia coli*, and *L. monocytogenes* were some of the bacteria isolated. They had the following percentage frequencies of occurrence: 40.20, 13.80, 11.50 and 8.04 respectively. *L. monocytogenes* contributed to the burden of spontaneous abortion in Jos and environs.

Key words: Spontaneous abortion, *Listeria*, isolation, incidence.

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Introduction

Spontaneous abortion and miscarriage are terms that are synonymous. It is the termination of pregnancy between 20 weeks gestation or the delivery of a foetus weighing less than 500 g (Shiers, 2001). Bacterial infections were reported as major causes (Smith et al., 2009). This was supported by the isolation of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, *Bacillus* spp and *Listeria monocytogenes* from aborted placenta bits by Mirdamadi (2005) and Kaur et al. (2007) in some cases of spontaneous abortion. The severity of Spontaneous abortion was seen in the high mortality and morbidity recorded in neonatal deaths as well as pregnant mothers, as observed by Seale (2009).

L. monocytogenes is a gram positive coccobacilli. It is a psychrophile and can grow at refrigeration temperature. It is a food borne pathogen reported to contaminate foods such as vegetables, raw and unpasteurized milk and cheese (Anonymous 2000); the foetus of a pregnant mother who ingested such contaminated food could be infected through the gastro-intestinal tract route during systemic illness (Smith et al., 2009). In addition, 16 serotypes of *L. monocytogenes* have been identified. Serotypes 1/2 a, 1/2b and 4b were responsible for 90% of Listeriosis cases world wide (Elcuaz et al., 1996).

Furthermore, person-to-person transmission of *L. monocytogenes* was reported to be possible through venereal contact as the bacterium was isolated from human semen (Toaff et al., 1962). In addition, Onyemelukwe et al. (1983), isolated *L. monocytogenes* from urethral swabs from males who patronized prostitutes in Zaria, north western Nigeria. This establishes the possibility of a pregnant mother being infected with *L. monocytogenes* through sexual intercourse, which could lead to spontaneous abortion. However, the data on spontaneous abortion in Africa, caused by *L. monocytogenes* are extremely rare (Anonymous, 2000). This could be attributed to the fact that when patients present with cases of spontaneous abortion, medical doctors do not directly request for diagnosis

of *L. monocytogenes*. Since it may require more than 24 hours to grow on a culture medium, laboratory scientists may report negative results, especially in cases of spontaneous abortion (Campbell, 1990).

Maternal mortality in northern Nigeria is among the highest in the world (Henry et al., 2011). Chiwuzie et al. (1995), working on causes of maternal mortality in a semi-urban Nigerian setting had earlier observed that globally, 500,000 women died during pregnancy and childbirth yearly, most of whom are from developing countries. Bacterial infections was one of the major causes. In Jos, central Nigeria, out of the 9,056 women delivered, genital tract sepsis accounted for 16.7 % of the causes (Mutahir et al., 2011). Consequently, knowledge of the involvement of other bacteria may be useful in the correlation of instances involving *L. monocytogenes*, in order to obtain an overall picture of the role of this organism in spontaneous abortion in Jos.

Materials and Methods

One hundred placental samples (bits) and 100 blood samples were obtained from each female patient voluntarily in three hospitals in Jos. The samples were preserved in a refrigerator at 4°C. They were categorized into six age groups (years) that consisted of 15-19, 20 – 24, 25 – 29, 30 – 34, 35 – 39, and ≥ 40. Patient consent was sought before samples were collected.

Treatment of Samples: Each placental sample was inoculated aseptically into 3.7g /100 ml (w/v) Brain Heart Infusion (BHI) broth (Difco, Michigan) and 3.2g / 100 ml (w/v) *Listeria* enrichment broth (Difco, Michigan). The broth cultures were incubated at 37°C for 24 hours and examined for growth. A blackening of the *Listeria* enrichment broth medium was noted as evidence of growth as described by Curtis and Lee (1995). The blood samples were subjected to the same treatments given to the placental samples. All the media were examined for evidence of growth. Furthermore, cold enrichment procedure and subsequent sub-culture on to listeria selective agar (Difco, Michigan) was also carried out forth nightly after which samples that did not show evidence of growth were reported as negative. All presumptive isolates were identified by standard microbiological methods as described by Cowan and Steel (1992).

Serology: The *Listeria* genus was identified to a particular serotype by serotyping it with Bacto-*Listeria* – O antisera supplied by Difco Laboratories Ltd., Detroit, Michigan.

Control culture: A *L. monocytogenes* isolate (SLCC 2379 Wurzburg) was similarly inoculated on the test media. This served as a control culture.

Results

Eighty seven (87) placenta swab and 13 blood samples were culture positive for eight confirmed species of bacterial pathogens (Table 1). *Enterococcus faecalis* was the most isolated pathogen, followed by *Proteus mirabilis*, *E. coli* and *L. monocytogenes* respectively. In addition, *L. monocytogenes* and *Streptococcus agalactiae* were also isolated from blood samples. Infection in the age range of the patients showed that *L. monocytogenes* was isolated in the age range 30 – 34, 20 – 24, 25 – 29 respectively while the age range 15 - 19 had the highest number of bacterial pathogens isolated other than *L. monocytogenes*. Details are in Table 2.

Table 1: The Number and Percentage of Species of Micro –organisms Isolated from Placenta and Blood Samples.

Species of Microorganisms	Placenta	Blood	Total (%)
<i>Enterococcus faecalis</i>	35	-	35 (40.20)
<i>Proteus faecalis</i>	12	-	12 (13.80)
<i>Escherichia coli</i>	10	-	10 (11.50)
<i>Listeria monocytogenes</i>	7	7	14 (8.04)
<i>Staphylococcus aureus</i>	10	-	10 (11.50)
<i>Staphylococcus agalactia</i>	6	6	12 (6.90)
<i>Candida albicans</i>	4	-	4 (4.60)
<i>Streptococcus pyogenes</i>	3	-	3 (3.45)
Total	87	13	100

Key: - represents no isolate

Table 2: Occurrence of Microorganisms (Isolated from Placenta and Blood) in the Age Range of the Patients

Microorganisms	Age Range (years)						Total
	15 - 19	20 - 24	25 - 29	30 - 34	35 - 39	≥40	
<i>Candida albicans</i>	3	1	-	-	-	-	4
<i>Escherichia coli</i>	6	-	-	-	4	-	10
<i>Listeria monocytogenes</i>	-	4	3	7	-	-	14
<i>Proteus mirabilis</i>	-	-	-	-	-	12	12
<i>Staphylococcus aureus</i>	10	-	-	-	-	-	10
<i>Streptococcus agalactiae</i>	-	-	-	4	-	8	12
<i>Enterococcus faecalis</i>	-	-	-	14	-	21	35
<i>Streptococcus pyogenes</i>	3	-	-	-	-	-	3
Total	22	5	3	25	4	41	100

Key: - represents no isolates

Serological investigation on the 14 *L. monocytogenes* isolates showed that 4 of the isolates were of serovar 1a, while 10 were of serovar 4b.

Discussion

The 8 species of pathogenic bacteria (*E. faecalis*, *P. mirabilis*, *E. coli*, *L. monocytogenes*, *S. aureus*, *S. agalactiae*, *C. albicans* and *S. pyogenes*) isolated from the cases of spontaneous abortions were significant, as they stem from specimens taken from the patients. This agrees with one of Kock's postulates, that the organism should be grown artificially in pure form from the tissue (Potter et al., 1968). Thus, since these organisms were isolated in pure form in a case of an infection, they could be considered the cause of the disease condition. This result showed that *L. monocytogenes* was not the only cause of spontaneous abortion in the subjects and agrees also with the findings of Kaur et al. (2007); Frietag et al. (2009) and Smith et al. (2009).

Although eight species of bacteria were isolated, only two species, *L. monocytogenes* and *Streptococcus agalactiae*, were isolated from blood and placenta indicating that there was spontaneous abortion secondary to septicaemia (Jensen & Andersen, 1979). The role of the other bacterial pathogens in spontaneous abortion may be due to genital tract infection (Dashner, 1997). This also agrees with the findings of Onyemelukwe et al. (1983) in which they isolated *L. monocytogenes* from the vaginal swabs of non-pregnant adults.

A view of the age groups affected showed that *L. monocytogenes* and *S. agalactiae* affected patients in the age group, 20-24, 25-29, 30-34 and ≥ 40 years, respectively. *L. monocytogenes* did not affect the age group ≥ 40 years. The age range 30-34 years suffered more from spontaneous abortion caused by both *L. monocytogenes* and *S. agalactiae* while spontaneous abortion in the age range 25 – 29 caused by bacteria other than *Listeria* could be the result of infection through the genital tract route as reported by Mutihir et al. (2011) in Jos central Nigeria.

Serological investigation on the *L. monocytogenes* isolates revealed that serovars 1a and 4b were responsible for the cases of spontaneous abortion in Jos. The results obtained confirm the earlier findings of Elcuaz et al. (1996); Onyemelukwe et al. (1983) and Sirry et al. (1994). The occurrence of types 1a and 4b of *L. monocytogenes* in Jos suggests the pathogenic potential of the said strains of microorganisms (Ozturk, 1990).

This showed that *L. monocytogenes* and other species of micro-organisms were associated with spontaneous abortion in Jos. A detailed surveillance study covering the entire state will give an overall picture of the involvement of these organisms in spontaneous abortion, while disease surveillance and awareness campaign would help monitor the trend of infection.

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